Amendments to the Claims

Applicants have submitted new claims drawn to methods of cancer diagnosis, as described at page 4, lines 14-17, and exemplified in Example 7.

The amendments to the claims do not introduce new matter. Applicants request entry of the amendments into the record.

The Rejection of Claims 8-14 under 35 U.S.C. §112, first paragraph

Claims 8-14 were rejected under 35 U.S.C. §112, first paragraph, as not enabled. Applicants traverse for the reasons set forth below.

Claims 8-14 are drawn to a method for quantitating telomerase activity in a human sample based on quantitating hTERT mRNA in the sample using the methods of the present invention. The specification teaches that hTERT mRNA encoding the active hTERT protein is a surrogate marker for telomerase acitivty (page 3, lines 15-19), and that the "quantity of hTERT mRNA, when calibrated as described herein, provides an estimate of the telomerase activity" (page 4, lines 10-11, emphasis added). The specification also provides experimental results demonstrating a strong correlation between telomerase activity and hTERT mRNA level using 8 different immortal cell lines (see Example 5 and Figure 3).

Examiner suggested the claims are not enable because the correlation may not be seen in all tissues, and cited art that teaches several studies "in which the hTERT mRNA level does not fully correlate to telomerase activity" (Office action, page 4, emphasis added). Applicants respectfully assert that the rejection represents an improper standard of enablement. Below, Applicants discuss the cited references and show that the rejection of the claimed methods is improper and should be withdrawn.

Nakamura et al.

Nakamura et al. (herein "Nakamura") examined telomerase activity in gastrointestinal tissues using a version of the TRAP assay, and also looked at hTERT mRNA levels. Nakamura reported that telomerase activity did not always fully correlate with hTERT mRNA in gastrointestinal tissue. Nakamura contrast their finding in gastrointestinal tissue with previously reported finding using other tissues (page 312,

second column), and with their own findings using hepatic tissue ("In hepatic tissues, there was a clear correlation between the hTERT expression level and telomerase activity..." page 319, column 2, lines 7-9; "...intrinsic characteristics of gastrointestinal tissues (which are different from hepatic tissues) .....and that the telomerase activity in gastrointestinal carcinomas did not always fully correlate with the hTERT mRNA level" page 320, last sentence before acknowledgements). Thus, Nakamura taught that gastrointestinal tissue is anomalous.

Applicants believe that the conclusions drawn by Nakamura are not supported by the results reported, and that this would be clear to one of skill in the art. In particular, Applicants believe that Nakamura clearly mischaracterized the RT/PCR assay used for the measurements of hTERT mRNA in at least two respects and, as a result, the reported correlation results are not valid. First, although Nakamura reported that the RT/PCR used did not amplify the β-deletion mRNA transcripts (see page 314, column 1, last sentence), this is not plausible in view of the description of the assay[1]. The primers used clearly should amplify the β-deletion mRNA transcripts, and the presence of β-deletion mRNA transcripts could render estimates of the correlation between mRNA transcripts and telomerase activity unreliable. This is particularly true given that splice variants account for a significant fraction of total hTERT transcripts (e.g., see Ulaner et al., cited

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[1] Nakamura amplified hTERT mRNA using primers U1513 and L1982, whose sequences are provided at page 314, column 1. By inspection, Applicants have determined that these primers hybridize to nucleotides 2175-298 and 2644-2667 of the sequence provided in the specification at pages 8-10. For Examiner's convenience, the relevant portions of the hTERT sequence are shown below with the positions of the primers indicated.

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2161 gccgcctgag ctgtactttg tcaaggtgga tgtgacgggc gcgtacgaca ccatccccca
                  5' |-----U1513--------------| 3'

2641 gctcctgcgt ttggtggatg atttcttgtt ggtgacacct cacctcaccc acgcgaaaac
       ||||||| |||||||||| |||||||
     3'ggacgca aaccacctac taaagaa 5'
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These primers hybridize to regions which correspond to the exon 5/6 junction and to exon 10 (see the specification at page 10) and, therefore, would amplify a region spanning the β deletion (i.e., exons 7 and 8). Although Nakamura suggests that these primers did not amplify the β-deletion mRNA transcripts (see page 314, column 1, last sentence), Applicants believe that it would be obvious to one of ordinary skill in the art that this statement has no basis and is incorrect, based on the position of the primers.

below, at page 4171, column 2), and that the deletion transcript, being shorter, typically would be preferentially amplified in an RT/PCR, which would make detecting a positive correlation even more difficult. Second, although Nakamura describes the results of the RT/PCR assay as a quantitative measurement of hTERT mRNA, Nakamura states that both the RT/PCR and the image analysis of gel fluorescence upon which the supposed quantitative result are based were not quantitative (page 316, column 2). For these reasons, Applicants believe that one of ordinary skill in the art would conclude that the results reported in Nakamura do not support any conclusions about a quantitative correlation.

Even assuming, *in arguendo*, the conclusions of Nakamura are valid, Nakamura only teaches that "the telomerase activity in gastrointestinal carcinomas did not always fully correlate with the hTERT mRNA level" (page 320, last sentence of the discussion, emphasis added), and, furthermore, that this result is specific to gastrointestinal tissues. Such teaching does not indicate that the claimed methods are not enabled. The patent specification teaches that the determination of telomerase activity from the hTERT mRNA level is based on a statistical association (see Example 5 and Figure 3), which inherently admits variability within the data. Such statistical results are useful even if the data "did not always fully correlate." Furthermore, the results of Nakamura are specific to a particular tissue type and represents anomalous results. It is well recognized that the discovery of a non-working embodiment within the scope of a claim does not invalidate the claim, in particular when the non-working embodiment can be routinely identified. In the present case, one of skill would identify any anomalous tissue type during the experimental determination of the correlation coefficient, all carried out as described in the examples.

Examiner also cited Nakamura as teaching several problems with the validity of a telomerase assay, including the presence of inhibitors of Taq DNA polymerase (Office action, page 3, lines 6-9). Nakamura describe this as a problem specific to gastrointestinal tissues, rather than as a general problem, and, further, teach how to solve the problem by using diluted samples (see the Abstract, lines 6-9, and the discussion of inhibitors page 314, column 2 through page 316, column 2). The specification teaches preferred sample preparation and assay methods suitable for a number of tissue types

(Example 1), and teaches that, in general, suitable sample preparation methods are well known in the art (page 15, lines 22-26). The Nakamura reference teaches the existence of problems specific to gastrointestinal tissue and teaches solutions to these problems. Thus, the Nakamura references supports the teaching in the specification that suitable sample preparation methods are well known in the art.

Wu et al.

Wu et al. (herein "Wu") reported on the correlation of the expression of human telomerase subunits, including hTERT, with telomerase activity in normal skin and skin tumors. It should be noted that that Wu describes a non-quantitative, presence-or-absence type of correlation, also referred to as concordance, rather than the quantitative estimation of telomerase activity from a quantitative measurement of hTERT activity, as used in the present invention. Wu concluded that there is a strong correlation (i.e., concordance) between telomerase activity and hTERT mRNA expression ("We conclude that telomerase activity in skin tumors is strongly correlated with hTERT expression," page 2043, column 2, last paragraph, emphasis added; "Activation of telomerase activity in skin tumors is strongly correlated with hTERT expression," page 2038, Abstract section entitled "CONCLUSIONS"). The data are presented in Table 2, page 2043, wherein Wu concluded "Correlation between telomerase activity and hTERT expression is statistically significant ($P<0.001$)."

Examiner improperly characterized Wu as teaching the unpredictability of estimating telomerase activity from hTERT activity when, in fact, the authors themselves conclude that there is a strong correlation between hTERT activity and telomerase activity. This teaching of a strong correlation between telomerase activity and hTERT mRNA expression does not in any way suggest that the claimed methods of estimating telomerase activity based on a correlation with hTERT mRNA expression are not enabled.

Aogi et al.

Aogi et al. (herein "Aogi") describe a comparison of telomerase activity (measured by a TRAP assay), hTERT mRNA expression (measured by non-quantitative

RT/PCR), and CD44 expression as diagnostic tumor markers in lesions of the thyroid. Aogi did not report on a correlation between telomerase activity and hTERT mRNA expression, although inspection of table 1 at page 2793 shows a non-quantitative, presence-or-absence concordance in 18/30 samples. The primers used to amplify hTERT mRNA were in exons 3 and 4 (page 2792, column 1, 2nd paragraph), and would be expected to amplify a region from both full-length and β-deletion transcripts. Furthermore, Aogi reported that when a product was seen, it was always accompanied by a second product, and attributed this to alternative splicing (paragraph spanning pages 2972-2973). Thus, the results of Aogi would be expected to be subject to the inaccuracies caused by expression of splice variants.

Examiner quoted Aogi as teaching that the use of hTERT for accurate analysis may be premature because of the incomplete understanding of the gene structure and the patterns of hTERT mRNA splicing that may be important in the expression of the functional enzyme. However, as the claimed methods overcome the inaccuracies inherent in the methods of Aogi caused by the expression of splice variants, the teaching of Aogi, made without the benefit of the present invention, does not support the suggestion that the claimed methods are not enabled.

Applicants further note that Aogi does teach about the state of the art at the time of filing of the present application, and, thus, does teach that Applicants' solution to the problem of inaccuracy caused by alternative hTERT mRNA splicing was not obvious.

For the reasons discussed above, Applicants maintain that the rejection represents an improper standard of enablement. None of the cited references supports a rejection of the claimed methods for lack of enablement. Applicants respectfully request reconsideration and withdrawal of the rejection of Claims 8-14 under 35 U.S.C. §112, first paragraph, in view of the amendments and remarks.

The Rejections of Claims 1-7 and 15-16 under 35 U.S.C. §103

Claims 1-7 and 15-16 were rejected under 35 U.S.C. §103 as unpatentable over Kilian et al. or Ulaner et al. in view of the Hall et al., U.S. Patent No. 5,593,862). Applicants traverse for the reasons set forth below.

Examiner suggested that it would be obvious to one of ordinary skill to modify the method of Kilian or Ulaner to position the primers within the beta-exon deletion region, as taught by Hall" (Office action, page 6, lines 3-6). However, as discussed below, one of skill wound not be motivated to modify the method of Kilian or Ulaner by positioning the primers within the β deletion region. In fact, Kilian and Ulaner teach away from using a primer within the β deletion because this would not allow detection of the major splice variants expressed. Below, Applicants first summarize the teaching of the cited art and then show that the claims are not rendered obvious by the cited art.

Kilian et al.

Kilian et al. (herein "Kilian") described the isolation of the hTERT gene (designated therein hTCS1) and reported the detection of several splice variants of the expressed mRNA. Kilian suggested that the splice variants, in particular, the altered relative expression levels of the major transcripts, might be involved in regulations of this gene (page 2017, first column 1, last 2 paragraphs). Kilian used methods which involve amplification of a major portion of the hTERT mRNA and gel analysis, which permits analysis of the pattern of splice variant expression. Kilian fails to teach or suggest methods of quantitating hTERT mRNA, fails to teach or suggest using a quantitative measure of hTERT mRNA to measure telomerase activity, and fails to teach or suggest selectively amplifying only mRNA transcripts that have the β-region intact.

Ulaner et al.

Ulaner et al. (herein "Ulaner") studied hTERT mRNA expression and the regulation of telomerase activity. Ulaner looked at hTERT mRNA expression in two ways. In the first, hTERT mRNA was amplified using primers that amplify a region upstream of the reverse transcriptase domain (page 4170, column 1), which is well upstream of the α and β regions (page 4169, Fig.1), and compared the results to

telomerase activity as measured by the TRAP assay. In the second, hTERT mRNA was amplified using primers that span both the α and β regions (Figure 1), followed by electrophoretic analysis of the amplified product, which permits analysis of the pattern of splice variant expression. Ulaner concluded that telomerase activity is regulated by at least two mechanisms: transcriptional control of the hTERT gene and alternate splicing of hTERT transcripts. It should be noted that Ulaner defined a full-length transcript as having a region encompassing both the α and β regions (Fig. 1). Ulaner fails to teach or suggest methods of quantitating hTERT mRNA, fails to teach or suggest using a quantitative measure of hTERT mRNA to measure telomerase activity, and fails to teach or suggest selectively amplifying only mRNA transcripts that have the β-region intact.

Hall et al.

Hall et al. (herein "Hall") relates to the isolation of genomic fragments for *Drosophila melanogaster* (a fruit fly) encoding a protein required for expression of functional voltage dependent cation channels. In an example, to confirm the existence of a deletion caused by a gene rearrangement, Hall amplified genomic DNA using primers within the deletion region. By amplifying both the DNA suspected of containing the deletion and a control DNA not having the deletion, Hall was able to confirm the presence of the deletion by the failure of the amplification. Hall fails to teach or suggest selectively amplifying full-length mRNA transcripts by using a primer that would fall within deletion in a splice variant.

Claims 1-7 and 15-16 are not rendered obvious by the cited references

Claims 1-7 are drawn to methods for quantitating hTERT mRNA. One of the critical aspect of the methods is the use of a primer that hybridizes within exon 8, which is a particular sub-region within the β-region. This aspect is not taught or suggested by the cited references. In fact, the cited references teach away from using such a primer by teaching a utility which would not be realized if a primer within the β-region were used.

In order to maintain an obviousness-type rejection, the cited art must not only provide the elements of the claimed invention, but there must be motivation to modify the prior methods to achieve the claimed invention. One of skill would not be motivated to

modify a prior art method in the case that the modification results in the method no longer having utility. In the present case, one of skill would not be motivated to modify the methods of Kilian or Ulaner by using a primer that hybridizes to exon 8 (within the β-deletion) because the resulting amplification would destroy the utility of the methods taught by Kilian and Ulaner.

Both Kilian and Ulaner teach non-quantitative analyses of hTERT mRNA carried out by amplifying a region spanning one or more regions deleted in splice variants and visualizing the products by gel electrophoresis. The methods enable the determination of the relative expression of different splice variants which involve a deletion within the amplified region. Furthermore, both Kilian and Ulaner teach that the relative expression of splice variants is involved in regulation and, thus, motivate one of skill to determine the relative expression of the splice variants. The use in the methods of Kilian or Ulaner of a primer that hybridizes to exon 8 would preclude a determination of the relative expression of the different splice variants and, thus, destroy the utility of the methods taught by Kilian and Ulaner.

Modification of the methods of Kilian or Ulaner in a manner that would eliminate the described utility would be contrary to the teaching of Kilian and Ulaner. Hall fails to provide a motivation to make such a modification. Thus, one of skill would not be motivated to make such a modification. Applicants respectfully urge that Examiner has improperly relied, using hindsight, on Applicants' specification for the motivation to modify the methods of Kilian and Ulaner to achieve the claimed invention.

Claim 2 is further distinguished by a the use of a primer in exon 6, in addition to the primer in exon 8. As taught in the specification, these primers provide particular efficient amplification of hTERT mRNA (see, e.g., page 16, lines 6-10). Neither Kilian or Ulaner teach or suggest that amplification of this particular region enables improved quantitation methods, and that this region alone provides a good surrogate marker for the expression of the full-length transcript. Note, in contrast, that Ulaner teaches the importance amplifying a region encompassing both the α and β regions, and thus teaches to use primers upstream of exon 6 and downstream of exon 8.

Claims 5-7 are further distinguished by the use of a particular pair of primers, one from exon 6 (selected from two) and one from exon 8, SYC1097 (SEQ ID NO: 4). Claim

15 is drawn to SYC1097. Claim 16 is drawn to a particular pair of primer, one of the two from exon 6 and SYC1097, from exon 8. Examiner suggested that the particular primers are obvious in view of the hTERT gene sequence because they "simply represent structural homologues of the full length disclosed hTERT sequence..." (Office action, page 7). The MPEP summarizes when a rejection based on structural homology is appropriate:

> A *prima facie* case of obviousness may be made when chemical compounds have a very close structural similarities and similar utilities. "An obviousness rejection based on similarity in chemical structure and function entails the motivation of one skilled in the art to make a claimed compound, in the expectation that compounds similar in structure will have similar properties"

(MPEP 2144.09, emphasis added, cites omitted). However, in the present case, neither structural similarity nor similar utilities is present.

For example, the primer SYC1097 is an oligonucleotide of length 18 having a specified sequence. In contrast, the full length hTERT sequence (SEQ ID NO: 1 at pages 8-10 of the specification) represents the sequence of a single-stranded mRNA[2] comprising 3961 nucleotides. The sequence of the hTERT mRNA does not even contain the sequence of primer SYC1097 as a subsequence, but rather contains the reverse complement of SYC1097 as a subsequence. As chemical compounds, these two molecules are vastly different and possess vastly different utilities. For example, the full-length hTERT mRNA cannot but used to amplify a target sequence within the full-length hTERT mRNA, and primer SYC1097 does not encode the hTERT protein. The presumption of obviousness based on structural similarity is overcome where there is no reasonable expectation of similar properties (MPEP 2144.09).

Applicants further maintain that two primers having different nucleotide sequences are not structural homologues in the chemical sense, even though both may hybridize to different regions of the same chromosome. Two primers with different sequences, viewed as chemical compounds, differ functionally in their hybridization

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[2] SEQ ID NO: 1 also is a representation of the coding portions of a much larger molecule of double-stranded DNA, which comprises both the exons and introns of the hTERT gene, and which itself is actually a region within a chromosome.

properties, which is a critical property of primers in general, and in particular, the primers of the present methods. Again, the presumption of obviousness based on structural similarity is overcome where there is no reasonable expectation of similar properties (MPEP 2144.09). Not only do the cited references fail to teach or suggest the particular primers recited in Claims 4-7 and 15-17, but these particular primers are not rendered obvious by the disclosure of primers which amplify other regions of the hTERT mRNA.

Applicants respectfully request withdrawal of the rejection of Claims 1-7 and 15-16 under 35 U.S.C. §103 in view of the remarks presented herein.

The Rejections of Claims 17-20 under 35 U.S.C. §103

Claims 17-20 were rejected under 35 U.S.C. §103 as unpatentable over Kilian et al. or Ulaner et al. in view of the Hall et al., as applied to Claims 1-7 and 15-17, in view of the Stratagene catalog. Applicants traverse for the reasons set forth below.

Claims 17-20 are drawn to kits which containing particular primers of the invention. Applicants showed above that the primer of Claim 15 and the primer pair of Claim 16 are not rendered obvious by the cited references. The kits of Claims 19 and 20 additionally containing probes which are not rendered obvious by the cited references. A kit containing nonobvious oligonucleotides is not obvious for the same reasons.

Applicants respectfully request withdrawal of the rejection of Claims 17-20 under 35 U.S.C. §103 in view of the amendments and remarks presented herein.

Conclusion

Applicants believe that all issues raised in the Office action dated August 28, 2000, have been addressed and that the application is now in condition for allowance. Applicants respectfully request reconsideration and withdrawal of the rejection of Claims 8-14 under 35 U.S.C. §112, first paragraph; the rejection of Claims 1-7 and 15-16 under 35 U.S.C. §103; and the rejection of Claims 17-20 under 35 U.S.C. §103; in view of the amendments and remarks presented herein.

Respectfully submitted:

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